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Deoxyribonucleic Acid Repair in Bacteriophage

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INTRODUCTION

Bacteriophage studies have provided considerable insight into the kinds of deoxyribonucleic acid (DNA) repair which exist in nature and into the genetic mechanisms involved in repair. Phage T4 and λ repair systems, especially, have been characterized genetically, enzymatically, and with respect to the relevant intracellular DNA configurations. Although DNA repair in bacteria and mammalian cells has been reviewed recently (for example by Smith [176], Hanawalt et al. [65], and Hart et al. [71]), there has been no comprehensive review of repair among phages since the review by Stent in 1963 (179).

The first early suggestion of a possible repair process after ultraviolet (UV) irradiation was by Hollaender and Curtis in 1935 in their work with *Escherichia coli*. This report was reprinted in

1975 (77) for historical interest. However, the earliest report in which recovery from potentially lethal damage was specifically postulated to be due to a repair or reactivation process was in 1947 by Luria (110). Using the phage T4 system, Luria investigated the phenomenon of multiplicity reactivation (MR) and proposed that this reactivation could be due to a type of recombination. In 1948 Kelner found photoreactivation of UV-treated conidia of *Streptomyces griseus* and communicated this result to Dulbecco. A few weeks later Dulbecco found photo-reactivation in UV-treated T phage. Both Kelner (91) and Dulbecco (44) published their work in 1949. Studies on excision repair began with the observations by Luria in 1947 (110) and Luria and Dulbecco in 1949 (112) that phage T4 is about twice as resistant to UV irradiation as phages T2 and T6. In 1949 this resistance was shown to be due to a single genetic unit by Luria (111), and this result was confirmed by Streisinger in 1956 (182). Thus, historically, some of the earliest work on the three main known forms of DNA repair (recombinational repair, photoreactivation, and excision repair) was done with phage.

In this review I describe the various types of lesions which are subject to repair in phage, the enzymes thought to be involved in these repair processes, the several distinct types of mechanisms employed, and the probable DNA structures formed as intermediates in repair. I also discuss some evolutionary implications suggested by our current understanding of phage DNA repair. In some cases where repair of phage DNA is carried out by bacterial enzymes, the process can be considered largely a reflection of bacterial, rather than phage, repair systems. These cases, which primarily involve phage λ, are summarized only briefly since authoritative, recent reviews are available and are referred to as appropriate. Considerable emphasis is given to phage T4 because of the large amount of information available on repair in this organism.

TYPES OF LESIONS REPAIRED

Summary of Agents Inducing Deoxyribonucleic Acid (DNA) Lesions and Effectiveness of Repair

Repair of DNA lesions is most often inferred from indirect lines of evidence. If a chemical agent or type of radiation is known to alter DNA structure and if upon treatment of phage or phage-infected cells it is found to cause decreased survival of the ability to form infective centers, it is usually inferred that DNA damage is the basis of the inactivation. Furthermore, if a phage mutant is found to be more sensitive to the agent than the wild type, then it is usually assumed that the wild-type function that is missing in the mutant participates in repair of the lesion.

Repair in single infections. The inactivating agents which have been used with phage T4 and have been studied most are UV irradiation, psoralen plus near-UV irradiation (PUVA), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), methyl methane sulfonate (MMS), mitomycin C (MMC), and nitrous acid. Table 1 shows for each of these agents the relative numbers of lethal lesions delivered to various mutant phages per lethal lesion delivered to the wild type by the same treatment. This is a measure of the relative sensitivity of each mutant to the inactivating agent. All values shown are for single infections (that is, where survival of infective centers is measured under conditions in which only one phage particle infects each cell). This table indicates the 14 genes whose products appear to be utilized in repair by this test, the alleles tested, and the relevant references. The relative numbers of lethal lesions delivered to mutant phages varies from 1.0 (no increased sensitivity) to 3.4. The value of 3.4 was obtained for the gene 47 mutant *ts*L86 treated with MNNG. By using this value, it can be calculated that when the gene 47 product is functional, (3.4 − 1.0)/3.4 or 71% of the lesions which occur in this mutant are repaired. Calculations such as this could underestimate the fraction of lethal lesions which are reparable, since another repair pathway, not involving the tested gene function, might remove additional lesions caused by this agent. The figure of 71% could also be an underestimate because the mutant used in the example, *ts*L86(gene 47), is a temperature-sensitive (*ts*) mutant, as are many of the mutants listed in Table 1. Testing of viability after treatment with an agent must be done at a temperature which is not completely restrictive, in order to allow survivors to form infective centers. Thus, the decreases in survival that are measured after treatment are made under conditions in which there is residual gene function. Using the data from Table 1 for the mutant most sensitive to each agent, we can calculate that the minimum percentages of potentially lethal lesions which are repaired are 71% for MNNG, 66% for MMS, 55% for UV irradiation, 50% for PUVA, 41% for MMC, and 23% for HNO_2.

Multiple pathways of DNA repair can be inferred if there is greater sensitivity of a double mutant than of the corresponding single mutants. When there is only a single pathway for repair, interposing a second mutational block should have no increased effect beyond that exerted by the first such block, if the mutants

TABLE 1. *Numbers of lethal lesions which various agents produce in phage T4 mutants per lethal lesion in wild type in single infections*[a]

Gene	Mutation	No. of lesions produced by:[b]					
		UV irradiation	PUVA	MNNG	MMS	MMC	HNO_2
Wild type		1.0	1.0	1.0	1.0	1.0	1.0
30	*ts*B20	1.2 (6)[c]	1.2[d]		2.9 (7)[e]		1.0 (17)
32	*ts*L67	1.4 (6)[c]		2.6 (155)		1.1 (78)	1.3 (136)
41	*ts*A14	1.1 (6)[c]					
	*uvs*58	1.2 (194)			1.4 (193)[e]		
	*uvs*79	1.2 (194)			2.1 (193)[e]		
43	*ts*L91	1.1 (6)[c]					
		1.0 (156, 178)					
	*ts*CB120	1.0 (217)	1.2 (217)[e]				
46	*ts*C17	1.4 (6)[c]		3.0 (155)		1.4 (78)	
	*ts*L109	1.0 (6)	1.5[d]	3.1 (155)			1.2 (136)
47	*ts*L86	1.2 (6)[c]	1.5 (217)[e]	3.4 (155)		1.7 (78)	1.3 (136)
	*ts*A52	1.2 (6)[c]					
58–61[f]	*m*6	1.4 (64)					
	*m*28, *m*57	1.5 (64)					
	*am*E219, *m*52, *m*54	1.6 (64)					
	*m*19	1.7 (64)					
	*m*29	1.8 (64)					
59	*am*C5	1.6 (212)[e]			2.8 (212)[e]		
	*am*HL628	1.9 (212)[e]					
*den*V	v_1	2.2 (68)[g]	1.0 (221)	1.0 (155)	1.0 (132, 192)	1.0 (78)	1.0 (70)
	*am*5	2.2 (194)					
	*uvs*52	1.4 (192)			1.0 (192)		
mms	*mms*1	1.1 (48)			1.4 (48)		
*uvs*W	*m*22	1.5 (64)	1.4 (221)[i]		1.8 (64)[e]		
	*m*33	1.5 (64)					
	*m*107	1.5 (38)					
	1206[h]	1.2 (185, 191)	1.1 (62)				
*uvs*X	*x*	1.7 (69)[g]	1.6 (221)	1.9 (155)	1.8 (48, 64)	1.5 (78)	1.2 (70)
y	*y*	1.7 (27)[g]	1.6 (221)	1.6 (155)	1.9 (48)	1.5 (78)	1.2 (70)
polA (host)	P3478	1.3 (121)[e]			1.6 (48)		
		1.2 (192)					
		1.1 (132)[e]					
	JG138	1.0 (132)	1.0 (221)				

[a] When applied to free phage or to phage-host complexes, all agents caused inactivation of the ability to produce infective centers. In general, when the log of the surviving fraction was plotted versus the dose of the agent, the survival curve was a straight line, although in the case of some UV irradiation curves there was a very small initial shoulder; that is, except for these few small shoulders, survival curves represented killing with single-hit kinetics, following the equation $N/N_0 = e^{-kd}$, where N_0 is the number of phage present in an initially chosen population, N is the number of surviving phage after treatment, d is the dose of the inactivating agent, and k is the number of lethal lesions introduced per unit dose. The numbers in the table represent $k_{mutant}/k_{wild\ type}$. The values listed were taken directly from the indicated reference, except as noted below in footnote *e*.

[b] The numbers in parentheses are reference numbers.

[c] These numbers of lethal lesions were calculated from values given in reference 6 as 0.1% survival dose ratios.

[d] These data are from V. Johns (personal communication).

[e] These values were calculated for the purpose of this table from curves presented in the references.

[f] Mutants of gene 58–61 were originally misclassified into two complementation groups, corresponding to genes 58 and 61. Subsequently, however, it was recognized (219) that these mutations belong to one cistron, and the gene was then designated 58–61 (211).

[g] The sensitivities of *v*, *x*, and *y* to UV irradiation have been evaluated in many laboratories. Only the first reported values are given here. All other values are very close to or the same as the original ones. The strain *x* contains other mutations, but these do not affect the UV sensitivity (64).

[h] Strain 1206 contains multiple mutations (191), including *m*107 of gene *uvs*W (38).

[i] It is not clear which mutation in gene *uvs*W was used in this experiment.

are not leaky. Conversely, if a double mutant containing two nonleaky mutations has increased sensitivity beyond that of each single mutant, the mutants are considered to be defective in two separate pathways. Using these criteria, investigators in two laboratories have reported three independent repair pathways for UV-induced damage. In one laboratory the three pathways are defined by the mutant *am*5(*den*V) (the gene in which the mutation is located is given in parentheses) in the excision repair pathway, the mutants *x*(*uvs*X) and $y_{10}(y)$ in an *x*-*y* pathway, and the mutants *uvs*58(gene 41) and *uvs*79(gene 41) in a third pathway (39, 193, 194). In the other laboratory the three pathways were defined by mutants *v*(*den*V), *x*(*uvs*X) and *y*(*y*), and *mms*1(*mms*) (48). It was shown (194) that the triple mutant *am*5(*den*V) $y_{10}(y)$ *uvs*79(gene

41) incurs 4.6 lethal lesions after a UV exposure which causes only 1.0 lethal lesions in the wild type. Thus, (4.6 − 1.0)/4.6 or 78% of the UV-induced lethal lesions are repaired by the combined action of all three pathways in a wild-type single infection. It can be calculated from the data in Table 1 and from the relative number of 4.6 lesions delivered to the triple mutant by UV irradiation that the levels of repair contributed by the individual pathways are (2.2 − 1.0)/4.6 or 26% by the *den*V pathway, (1.7 − 1.0)/4.6 or 15% by the *x-y* pathway, and (1.2 − 1.0)/4.6 or 4% by the gene 41 pathway. Compared with the 78% of all lesions repaired calculated above, the combination total of 45% suggests that the pathways may compete for some of the same lesions. For instance, when the *x-y* pathway is defective and lesions are left that would usually be repaired by this pathway, one of the other pathways may repair some of these lesions.

Repair in multiple infections. When two or more damaged phage chromosomes enter a host cell, MR can occur. In 1947, Luria (110) was the first to study this phenomenon systematically. He treated phage T4 or T6 with doses of UV irradiation that reduced the fraction of phage able to survive and form infective centers in single infections to 10^{-3} to 10^{-4} of the untreated population. When these treated phage were allowed to infect cells at different multiplicities between 0.01 and 0.95, the number of infective centers obtained was close to the calculated number of doubly infected cells. Luria concluded that reactivation of UV-inactivated particles could occur inside bacterial cells that adsorbed two or more phage particles. In 1952, Dulbecco (46) performed a careful quantitative study of this phenomenon and concluded that it could be due to repair occurring in the multiply infected cells.

Generally, the data from MR experiments are presented by plotting the log of surviving infective center-forming ability of multiply infected cells (multicomplexes) versus the dose of inactivating agent. These multicomplex inactivation curves are usually straight lines or straight lines with an initial shoulder, depending on the agent used and the mutations present in the phage. Over the straight-line portions of these curves, the inactivation kinetics can be represented in a form similar to the form of the inactivation kinetics of singly infected cells. Thus, multicomplex inactivation over a straight-line portion follows the equation $N/N_0 = e^{-kd}$, where N_0 is the number of multicomplexes in an initially chosen population, N is the number of surviving multicomplexes after treatment, d is the dose of the inactivating agent, and k is the number of lethal lesions delivered to the multicomplex per unit dose.

When a comparison is made of the survival of infective center-forming abilities of singly infected cells (monocomplexes) and multiply infected cells (multicomplexes), a term called the MR factor can be calculated. The MR factor for any strain is given by k_{mono}/k_{multi}, where the k values are taken from the straight-line portions of the curves. This factor is a measure of the strength of the MR effect. Table 2 shows the MR factors of wild-type phage after treatment with each of nine different agents. This table shows that the highest observed MR factors, 10 and 8.5, were obtained after treatment with MMC and PUVA, respectively. Treatment with HNO_2, ^{32}P, UV irradiation, X rays, or MNNG gave MR factors in the range of 5.0 to 2.9, and treatment with ethyl methane sulfonate (EMS) or acridine orange gave an MR factor of 1.0.

For three of the nine inactivating agents listed in Table 2 (X rays, EMS, and UV irradiation), the multicomplex inactivation curves had substantial shoulders. In these cases, the shoulder may reflect the action of an additional MR process which can be saturated when a certain number of lethal lesions has been reached (136). This interpretation is favored since, as discussed below, the shoulder is eliminated in some repair-defective mutants. The length of the shoulder is defined by the dose of inactivating agent needed to reach the transition to the straight-line portion of the curve. This dose can be represented in terms of lethal hits delivered to monocomplexes (observed in experiments performed in parallel with the multicomplex experiment). Table 2 gives the amounts of shoulder repair measured in these terms.

The MR factor and the shoulder repair value together indicate the additional repair available in multicomplexes compared with monocomplexes. For MMC with an MR factor of 10 and no shoulder repair, 10-fold-fewer lethal lesions remain in an average multicomplex compared with the number present in an average monocomplex for any given dose of treatment. We can also present this type of information in another way. At a dose of MMC which reduces the survival of monocomplexes to 1% of their initial number, survival of multicomplexes is about 65% (78). For EMS-treated phage, where there is only shoulder repair, surviving monocomplexes can be reduced to 1% of their initial number at a dose which gives 50% survival of multicomplexes (88). For UV irradiation, there is both a large amount of shoulder repair and a significant MR factor. In the experiments of Dulbecco (46), inactivation of multicomplexes

TABLE 2. *Effectiveness of MR in overcoming lethal lesions introduced by various inactivating agents in phage T4*

Agent	MR factor[a]	Shoulder repair (no. of lethal hits)[b]	Reference
MMC	10	None	78
PUVA	8.5	None	Johns, personal communication
HNO_2	5.0	None	136
^{32}P	4.6[c]	None	186
X rays	4.0	27[c]	67
MNNG	2.9	None	155
EMS	1.0	6[c]	88
Acridine orange	1.0	None	37
UV irradiation[d]	2.3[c]	12[c]	37
	2.3	9	136
	2.3	12[c]	192
	2.4[c]	20[c]	68
	2.5	20[c]	66
	3.3[c]	30[c]	185
	3.4[c]	6[c]	49
	3.6	16[c]	27
	4.0[c]	25[c]	46
	5.0[c]	34[c]	191
	7.0	7-9[c]	216
	12.0[c]	19[c]	40

[a] The MR factor is equal to k_{mono}/k_{multi}, where k_{mono} is the inactivation constant for monocomplexes and k_{multi} is the inactivation constant for multicomplexes. These k values were calculated from the straight-line portions of the respective inactivation curves.

[b] Shoulder repair values represent the number of lethal hits, as determined from the straight-line portion of the monocomplex inactivation curve, which are delivered before the multicomplex inactivation curve also becomes a straight line on semilogarithmic coordinates.

[c] These values are calculated from data presented in the references.

[d] The data for MR of UV-induced lesions show considerable variability, but the source of this is not readily apparent. High values, similar to the two highest on the table, are expected of the UV-induced lesions which remain after photo-reactivation removes a fraction of the lethal lesions (46). It may be that photo-reactivation was a factor influencing the experiments with higher values. The average UV-induced MR factor was 4.2, and the average UV-induced should repair value was 18 lethal hits.

was barely measurable at a UV dose which reduced survival of monocomplexes to 10^{-5}. Thus, when MR occurs, it is often a dramatic phenomenon, reflecting a substantial increase in survival of multicomplexes compared with monocomplexes.

Thymine Dimers

Although Tables 1 and 2 list agents which cause reparable lethal lesions (except for acridine orange), they do not specify the chemical nature of the lesions. In the case of UV irradiation, a number of photoproducts are produced in the DNA. These include pyrimidine dimers (thymine-thymine, cytosine-thymine, and cytosine-cytosine dimers) (162), DNA cross-links (114), single-strand breaks (113), cytosine-thymine adducts (195, 202), cytosine hydrate (63), dihydrothymine (215), DNA-protein cross-links (174, 175), and other lesions which are less well characterized (51, 102). Meistrich (125) has shown that after UV irradiation of phage T4, thymine dimers (as distinct from all pyrimidine dimers) account for 56% of the lethal hits if the major repair pathways dependent on the products of *den*V and *uvs*X are absent. A rough comparison can be made with the work of Setlow and Setlow (164), who have estimated that in *Haemophilus influenzae* transforming DNA, about 50% of the biological inactivation effects of UV irradiation are due to thymine dimers. It is not clear to what extent repair occurs for transforming DNA, and it should be kept in mind that the proportion of lethality caused by thymine dimers varies with the extent to which the dimers are repaired.

Meistrich (125) showed that the double mutant having defects in both the *den*V repair pathway and the *uvs*X repair pathway had a ratio of 2.5 thymine dimers created per lethal hit to the phage. If we picture 10 such double-mutant phage which have been treated with UV irradiation at a dose which delivers an average of 0.2 lethal hit per phage, there may be one phage carrying a lethal thymine dimer, one phage carrying a lethal lesion which is not a thymine dimer (56% of the lethal hits in these phage are thymine dimers), and four phage each carrying a thymine dimer which is not lethal (there are a total of two lethal hits in our population, and we expect 2.5 thymine dimers per lethal hit, or a total of 5 thymine dimers). More accurately, we can infer from the data of Meistrich that 22% of all thymine dimers (56% divided by 2.5) induce lethality in the double mutant used. Thus, we conclude that in phage T4 either there are additional major repair pathways for thymine dimers in single infections or not all thymine dimers cause lethality. In addition, Meistrich (125) was able to show that the *den*V pathway preferentially repairs thymine dimers, whereas the *uvs*X pathway acts on all lethal lesions.

Pawl et al. (138) obtained evidence that not all thymine-containing dimers are acted on equally by repair processes after phage T4 infection. These authors showed that in a wild-type infection, about 50% of the thymine-containing dimers created in a phage chromosome could be

removed by repair during the first 5 min after infection. The remaining dimers were not removed with an additional 10 min of incubation. The result was the same whether DNA replication could begin or was inhibited.

Using UV-irradiated phage T7, Burck et al. (33) obtained evidence from an electron microscopic analysis and cross-reactivation experiments that DNA replication was blocked at thymine dimers. When photo-reactivation, which monomerizes pyrimidine dimers (184), was allowed to occur during infection, the blocks to replication were removed. Hourcade and Dressler (79) used a phage G4 system in which they could examine the conversion of an infecting positive single-stranded circle to a duplex ring. When these phage were first UV irradiated, only partially duplex strands were formed. The results of Burck et al. (33) and Hourcade and Dressler (79) indicate that in phages T7 and G4, DNA replication is blocked at the site of a thymine dimer.

Other Ultraviolet Photoproducts of Biological Importance

Lin and Howard-Flanders (102) showed with phage λ that UV irradiation induces at least two kinds of lesions which stimulate recombination. These are a photo-reactivable product (probably pyrimidine dimers), which only stimulates recombination after the DNA has been allowed to replicate, and a non-photo-reactivable 254-nm product, which stimulates recombination in the absence of replication. The second product constitutes a substantial fraction, perhaps 25 to 50% of the recombinogenic lesions.

In cells infected by UV-irradiated phage T4, the UV photoproducts left after photo-reactivation have a higher probability of undergoing MR than the combination of lesions present before photo-reactivation (46). As discussed below, MR of UV-treated phage T4 occurs by a recombinational mechanism. Thus, the non-photo-reactivable lesions in phages T4 and λ may be similar in stimulating forms of recombinational repair.

Alkylation Products

Alkylating agents produce a wide variety of alterations in DNA. A summary of these agents and the DNA alterations which they cause was given by Strauss et al. (181). With EMS, Lawley and Martin (96) reported that the proportions of ethylated purines in phage T4 were 51 to 66% 7-ethylguanine, 7 to 10% 3-ethyladenine, 1.5 to 3% 6-ethoxyguanine, and 0.6 to 2.4% 7-ethyladenine. Bannon and Verly (8) reported that 15% of all alkylations after EMS treatment are phosphotriesters. Several authors have speculated (e.g., Lawley and Martin [96]) that methylation or ethylation of DNA bases does not in itself cause phage inactivation, but that the spontaneous depurination of such bases or subsequent spontaneous chain scission may be the lethal event.

When wild-type phage T4 is treated with EMS at varying doses and then allowed to infect host bacteria, each inactivation curve (plotted on semilogarithmic coordinates) has a shoulder, followed by a straight-line exponential decline (31, 88). Approximately 500 ethylations of DNA must occur before any lethality is detected (31). This corresponds to the shoulder portion of the curve. In the exponential portion, where single-hit kinetics obtain, one can estimate from the curves presented by Brooks and Lawley (31) that there are about 400 additional ethylations for each lethal hit. It was shown in phage T4 that when mutations in gene 32, 46, or 47 are present, the shoulder of the inactivation curve is removed (88). With a mutation in phage gene *uvs*X (88, 146), *y*(88), or 30 (88, 146) or in a *polA* host (146), the shoulder is reduced. The remainder of the inactivation curve generally has a slope which is the same as or similar to the slope in the exponential portion of the wild-type inactivation curve. It can be speculated that the initial lesions introduced by EMS are subject to effficient repair by one or more pathways involving the products of phage genes 30, 32, 46, 47, *uvs*X, and *y* and the host gene *polA*, but that this repair system can be saturated by a certain number of lesions in the DNA. Beyond this point, additional lesions cause inactivation with single-hit kinetics.

Another alkylating agent about which a good deal is known is MNNG. Evidence has been presented that MNNG must be activated before it can cause either lethal or mutagenic lesions. Thus, phage λ DNA as present in mature phage particles has 3% of its bases methylated by an MNNG dose of 0.6 mg/ml, but the phage are barely mutagenized. On the other hand, intracellular phage λ DNA has 0.6% of its bases methylated by a dose of 0.09 mg/ml and is highly mutagenized (213). Similarly, phage T4 particles are not inactivated by MNNG, but when phage-host complexes are treated, the ability to form infective centers is inactivated (220). Also, mutagenesis occurs with MNNG treatment of phage T4 or phage S13 DNA once it is within a host cell, but does not occur with MNNG treatment of mature phage (4). Similarly, the *H. influenzae* phage Hp1c1 can be mutagenized with MNNG if phage-host complexes are treated, but there is no mutagenic effect of MNNG on free phage (22).

Lawley and Thatcher (97) found that thiols,

such as glutathione and *N*-acetylcysteine, which are expected to be present intracellularly, markedly enhance the rate of methylation of DNA by MNNG. The approximate percentages of methylated bases found in vivo (or in vitro in the presence of a thiol) were 73% 7-methylguanine, 10% 3-methyladenine, 7% 6-methoxyguanine, 2% 3-methylcytosine, and 1% 1-methyladenine. The major adduct, 7-methylguanine, is unstable in DNA and forms apurinic sites. These may hydrolyze, either spontaneously or through attack by a specific apurinic endonuclease, to produce single-strand breaks (103). The spectrum of methylated bases formed by MNNG treatment is somewhat similar to the spectrum of ethylated bases formed by EMS treatment. Which one or ones of these altered bases are the lethal lesions is not known. However, it has been suggested (213) that 6-methoxyguanine may be the mutagenic lesion produced by MNNG.

Phage T4-host complexes are inactivated by MNNG with single-hit kinetics (220). When a mutant defective in gene 32, 46, 47, *uvs*X, or *y* is used, single-hit inactivation is again observed, but the rate of inactivation in all cases is greater than the rate for the wild type (155). There was no initial shoulder on these MNNG inactivation curves, indicating that the repair pathway(s) coded for by these genes is not saturable, in contrast to what was found upon treatment with EMS.

In 1966, Loveless (109) summarized and interpreted the previously reported effects of alkylating agents on phages. He included a presentation of the shapes of the inactivation curves produced by the action of various agents on several kinds of phage under different conditions of treatment.

DNA Cross-Links

Many alkylating agents are bifunctional and induce DNA cross-links. In 1966, Loveless (109) comprehensively reviewed the quantitative inactivating effects of bifunctional cross-linking agents on phage.

Seki et al. (158) studied the effects of psoralen plus light on phage T7. Upon initial treatment with PUVA, both monoadducts and cross-links (diadducts) were formed in DNA. However, the monoadducts could be converted quantitatively to cross-links by additional light treatment if the unbound psoralen was washed away. These workers found that cross-links were more effective than monoadducts in causing phage inactivation. In a parallel study with *E. coli*, they also observed that the conversion of monoadducts into cross-links caused a loss of mutagenesis and an increase in lethality. This indicates that psoralen monoadducts are the more mutagenic lesions, whereas psoralen cross-links are the more lethal lesions.

Nitrous acid induces cross-links at a frequency which is about equal to the frequency of induction of lethal hits in phage T2 (10). However, nitrous acid also produces three oxidatively deaminated bases in DNA (hypoxanthine [from adenine], uracil [from cytosine], and xanthine [from guanine]) with sufficient frequency to also account for the lethality (196). Treatment of DNA with activated MMC in vitro produced lesions which were about 10 to 20% cross-links and 80 to 90% other products (187). In *H. influenzae* only 0.2 cross-link was introduced by MMC per lethal hit (172). Thus, in the case of MMC, cross-links may be lethal, but other adducts are lethal as well. MMC does not reduce survival of free phage, but it does reduce the infective center-forming ability of phage-host complexes (78, 160). Thus, MMC apparently must be activated intracellularly before causing lethal lesions.

In general, DNA cross-linking agents inactivate phage. They appear to produce products in addition to cross-links, and in most cases it is not clear whether the cross-link is the main lethal lesion. However, in the case of PUVA, where most of the lethal lesions should be cross-links (158), there is repair both in single infections (Table 1) and in multiple infections (Table 2). Thus, at least some cross-links undergo repair.

Mismatched Bases

In a review which included a discussion of the repair of mismatched bases in phage DNA, Radding (145) summarized evidence indicating that when this process occurs in phage λ DNA, it involves excision of a single strand segment of about 2,000 to 3,000 nucleotides and that, in addition, mismatch repair occurs with the *E. coli* phages φX174 and f1 and with the *Bacillus subtilis* phage SPP1.

In phage T4, mismatched bases are also corrected. This repair does not depend on the *den*V gene, which specifies endonuclease V, the enzyme that initiates repair of thymine dimers (13). However, when heteroduplexes are formed with small loops in one strand from pairing of a wild-type strand with a strand having a 1- to 5-base addition or deletion, these heteroduplex loops are removed by a repair system that does depend on *den*V (12).

Strand Breaks

When ^{32}P is incorporated into phage T4 DNA, radioactive decay produces both double-strand and single-strand breaks (128). In mature phage containing ^{32}P-labeled DNA, there is a good

correlation between the extent of inactivation and the frequency of double-strand breaks, suggesting that these lesions are lethal (98, 99). The single-strand breaks produced do not appear to be lethal in wild-type phage (98, 99) and are presumably repaired. Some of the inactivation by ^{32}P decay in mature phage may be due to interference with the injection process by the lesions, since if ^{32}P-labeled phage DNA is injected into host cells and decay is allowed to occur intracellularly, inactivation of monocomplexes occurs at a rate which is about 20% lower per ^{32}P decay than for free phage (186). If ^{32}P decay occurs within phage-host multicomplexes, some of the lethal lesions which occur are repaired by MR (Table 2).

Other Lesions

When phage λ was grown in the presence of 5-bromouracil (104) or phage T4 was grown in the presence of 9-aminoacridine (117), the recombination frequency measured with these phages was increased. Possibly this increase reflects recombinational repair of DNA alterations produced by these agents. However, as yet there is no direct evidence for repair of possible lesions caused by these chemicals.

Hypoxanthine and or uracil are two of the altered bases produced by the action of HNO_2 on DNA, and it is thought that one or both of these compounds are responsible for the mutagenicity of HNO_2 (196). This conclusion was based on experiments with phage T2 which showed that the change in the rate of production of these two products with varying pH values parallels the change in the rate of induction of mutation.

Childs et al. (35) irradiated phage T4 with 320-nm light. This created a 5-hydroxymethylcytosine photoproduct which could not be photo-reactivated. The photoproduct was repaired by the endonuclease V pathway, which also controls the excision repair of thymine dimers.

Irradiation of phage T4 by visible light in the presence of acridine orange causes inactivation, possibly either by elimination of a nucleotide base or by conversion of a base to a form having a labile *N*-glycosylic link (54). Acridine orange inactivation of phage T4 was not subject to MR, but an *r*II gene in phage inactivated by this treatment was subject to cross-reactivation (37).

PROTEINS INVOLVED IN REPAIR

Endonuclease(s)

Endonuclease V is the product of phage T4 gene *den*V (154). It cleaves the glycosylic bond at the 5′ half of a pyrimidine dimer (40a, 143, 157, 203) and subsequently catalyzes cleavage of the phosphodiester bond originally linking the two nucleotides of the dimer, leaving an apyrimidinic 3′ terminus in the newly nicked DNA (40a, 203). Mutants defective in *den*V are not more sensitive than wild-type phage to PUVA, MNNG, MMS, MMC, or HNO_2 (Table 1). Thus, endonuclease V does not appear to be required for repair of the lesions caused by these agents. Endonuclease V does not act on undamaged native or denatured DNA (218) but does efficiently nick depurinated DNA (40a, 203). When endonuclease V is added to nuclei from human xeroderma pigmentosum cells, which are otherwise unable to initiate repair synthesis in response to UV-induced lesions, the enzyme allows repair to proceed (173, 188). Endonuclease V can similarly reactivate permeable *E. coli uvrA, uvrB,* or *uvrC* mutants after treatment with UV irradiation (169).

Five other endonucleases, designated endonucleases I, II, III, IV, and VI, are coded for by phage T4 (211), but these are not known to be involved in DNA repair. Two of these, endonucleases II and IV, which are specified by genes *den*A and *den*B, respectively, are utilized in the breakdown of host DNA (75, 95, 204). The functions of the other three endonucleases are not known.

Exonuclease(s)

The products of two phage T4 genes, genes 46 and 47, are required for efficient repair of lethal lesions induced by UV irradiation, PUVA, MNNG, MMC, and HNO_2 in single infections (Table 1). Both gene functions may be replaced to a small extent by a host function, since all amber mutants defective in these genes produce characteristic small plaques on an Su^- host (130). The suppressor *das*, which maps in a separate gene, allows gene 46 and 47 amber mutants to produce high burst sizes in an Su^- host (76). The gene 46 and 47 products are also required for recombination (14, 18). When *ts* mutants defective in these genes are grown at semirestrictive temperatures that allow 5% of the wild-type burst size, recombination frequencies are reduced to 6 to 10% of the wild-type frequencies (18). Treatment of wild-type phage T4 with HNO_2 can increase the level of recombination by about sixfold above the spontaneous level, and this HNO_2-induced recombination is nearly all prevented by the presence of a gene 46 or 47 *ts* allele (58). These results suggest that the gene 46- and 47-controlled exonuclease(s) may be employed in a recombinational repair pathway.

Two DNA exonucleases, designated exonucleases A and B, have been identified in phage

T4-infected cells, but neither is known to have a DNA repair function. DNA exonuclease A was tested for involvement in host DNA breakdown, but no clear effect was found (205). DNA exonuclease B is able to catalyze the in vitro removal of thymine dimers from irradiated DNA which has been nicked, but it is not known whether it carries out this function in vivo (57, 159).

Phage λ exonuclease is coded for by the *red* gene (144, 170). It is responsible for a small amount of prophage reactivation of UV damage (41). The *red* gene product is also utilized in genetic recombination (144, 170).

Helix-Destabilizing Protein

The product of phage T4 gene 32 is a DNA helix-destabilizing protein (1). A mutant defective in gene 32 is sensitive to UV irradiation, MNNG, MMC, and HNO_2 in single infections (Table 1). Unlike most other proteins involved in DNA metabolism, the gene 32 protein is required in stoichiometric amounts, rather than catalytic amounts (177). It binds cooperatively to DNA and stabilizes single-stranded regions (2). It also interacts with the DNA polymerase encoded by gene 43 to promote efficient DNA elongation (84, 105). In addition to being required for efficient repair of lethal damage, as indicated in Table 1, it appears to interact with DNA polymerase in error-prone repair of UV-induced damage (134).

A gene 32 *ts* mutant which is defective in repair of lethal HNO_2-induced lesions is also defective in recombination, suggesting that the gene 32 protein may be involved in recombinational repair (136). From the configurations of the nonreplicating phage DNAs formed in the absence of the gene 32 protein, as observed by electron microscopy, it was proposed that the role of the gene 32 protein in recombination is to promote pairing of complementary single strands (30). This proposal is supported by the observation that the gene 32 protein facilitates pairing of complementary strands in vitro (3). It also appears to bind to single-strand termini, thereby blocking the 3′-to-5′ exonuclease activity of DNA polymerase (83), and this effect may also aid the pairing reaction. In addition, the gene 32 protein was found to enhance the in vitro uptake of homologous single strands by duplex DNA, when this process was promoted by the *E. coli recA* protein (168). It appears that the promotor-proximal region of the gene 32 protein affects DNA replication, whereas the C-terminal domain is required for control of nucleases employed in recombination (133).

DNA Polymerase

The phage T4 gene 43 product is a DNA polymerase that catalyzes DNA synthesis in a 5′-to-3′ direction (61), and it also has an exonuclease activity (61, 137) which acts in the 3′-to-5′ direction (83). This second function is thought to be used in the editing and proofreading of newly inserted bases (60). Mutants defective in gene 43 have been reported to have a decreased ability to repair UV-induced lethal lesions (Table 1) (120), although the possibility has been raised that the very small increase in sensitivity to UV irradiation caused by a gene 43 *ts* mutation may be due to a plating artifact (156). Gene 43 *ts* mutants also show small, but unambiguous, increases in sensitivity to PUVA (Table 1) and EMS (146, 156).

The gene 43 polymerase appears to have an essential role in error-prone repair carried out after UV or PUVA treatment. The gene 43 polymerase carries out replication in conjunction with five accessory proteins (the products of genes 32, 41, 44, 45, and 62) and a sixth protein, which is probably the product of gene 58–61 (105). Since mutational alteration of accessory proteins 32, 41, 44, and 45 can restore UV mutagenesis in the presence of an antimutator polymerase (134), error-prone repair synthesis may be carried out by the gene 43 polymerase in concert with these accessory proteins.

DNA Topoisomerase

A new group of DNA-related enzymes, designated topoisomerases, have been defined recently. The first of these is *E. coli* ω protein (topoisomerase I), which releases negative superhelical turns present in covalently closed DNA (201). A second enzyme, an *E. coli* gyrase, has been characterized (topoisomerase II), which can introduce negative supercoiling into DNA (59) and unknot knotted DNA (131). The properties of *E. coli* topoisomerase II have been reviewed extensively by Cozzarelli (36). The antibiotics coumermycin and oxolinic acid, which specifically inactivate *E. coli* topoisomerase II, were found to inhibit repair of UV-induced lesions and reduce recombination in phage λ (72). Phage T4 codes for its own type II DNA topoisomerase, which can unknot knotted DNA (107). This topoisomerase consists of the products of genes 39, 52, and probably 60 (106, 180). Mutants defective in genes 52 and 60 did not show increased UV sensitivity compared with wild type (64). However, mutants defective in these genes did show substantially reduced MR after treatment by PUVA or MMC, indicating that the topoisomerase may be needed for re-

combinational repair (V. Johns and S. Schneider, personal communication). The phage T4 topoisomerase is also utilized in initiation of DNA replication (122, 123).

DNA Ligase

The product of phage T4 gene 30 is DNA ligase (50). The gene 30 mutant *ts*B20 is significantly more sensitive than the wild type to UV irradiation and PUVA and substantially more sensitive to MMS (Table 1). In the presence of an *r*II mutation, the UV sensitivity of a ligase amber mutant is suppressed, possibly because the host ligase becomes more available for repair of the phage DNA (89). The gene 30 ligase appears to be required for error-prone repair of HNO_2-, UV-, and PUVA-induced lesions (17, 216, 217).

Other Phage Gene Products

Mutants defective in phage T4 genes 41, 58–61, 59, *uvs*W, *uvs*X, *y*, and *mms* are more sensitive than wild type to UV irradiation (Table 1). Some of these mutants also have increased sensitivity to PUVA, MNNG, MMS, MMC, and HNO_2. Mutants *uvs*58(gene 41), *uvs*79(gene 41), and *mms*1(*mms*) have similar phenotypes and could be defective in the same gene (39, 48, 194). Recently, the products of genes 41 and 58–61 were shown to be required for the initiation of Okasaki fragments by formation of ribonucleic acid primers (105, 171). The product of gene 41 also has a role in driving the leading strand at a replication fork (105). It has been suggested that the product of gene 41 may be involved in the formation of ribonucleic acid primers opposite DNA regions containing lesions (39). Gene 59 mutants are similar to gene 46 and 47 mutants in that they have a DNA arrest phenotype and have decreased recombination (165). The genes *uvs*W, *uvs*X, and *y* are similar to each other in some ways. Mutations in each of these genes reduce recombination (27, 38, 69, 191) and increase sensitivity to several damaging agents (Table 1). They also reduce UV- and PUVA-induced mutagenesis, presumably through a defect in an error-prone repair pathway (62). Since the double mutants *x*(*uvs*X) *y*(*y*) and 1206(*uvs*W) *y*(*y*) have about the same sensitivity to UV irradiation as the single mutant *y*(*y*), it appears that the *uvs*W and *y* gene functions operate in the same pathway for repairing UV-induced lethal lesions (26, 185). Mutations in genes *uvs*X and *uvs*W are not known to be conditionally lethal, so it appears that these genes do not code for essential functions (38, 64, 127). However, an amber mutation in gene *y* is lethal in an *Su*$^-$ host, indicating that gene *y* function is essential (119). A mutation in gene *uvs*W can partially suppress the deficiencies in recombination and UV resistance caused by a mutation in gene 59 (38). Additional properties of mutants with mutations in genes *uvs*X, *y*, and *uvs*W (38, 127) and gene 59 (38) are summarized in the references indicated.

Host Proteins

When treated with UV irradiation or MMS, wild-type phage T4 is more sensitive when it is grown in an *E. coli polA* host than when it is grown in a *polA*$^+$ host under single-infection conditions (Table 1), implying that the *polA* product has a role in repair of UV- and MMS-induced lesions in phage T4. This host function is also required for MR of UV-induced lesions in phage T4 (121). As reviewed by Kornberg (93), the *polA* gene specifies DNA polymerase I which, in addition to its 5′-to-3′ polymerizing function, has both 3′-to-5′ and 5′-to-3′ exonuclease activities. After infection by UV-irradiated phage T4, both the 5′-to-3′ exonuclease function and the polymerizing function of polymerase I are required for excision of pyrimidine dimers after an incision on the 5′ side by phage endonuclease V (138). Thus, the host polymerase I seems to be intimately involved in a repair pathway for phage DNA, whose initial step is controlled by a phage T4 gene.

The *E. coli recA* gene product is required for MR of UV-inactivated phage T4 (142). As discussed below, this MR is probably a recombinational repair process. A number of properties of the *recA* protein, which have been elucidated recently by West et al. (206), should be of importance in its recombination functions. These workers found that the *recA* protein binds cooperatively to single-stranded DNA or to duplex DNA containing single-stranded gaps. It also has an adenosine triphosphatase activity that is stimulated by gapped DNA. In reactions between the *recA* protein and gapped DNA, the presence of adenosine 5′-triphosphate resulted in the formation of DNA aggregates. It was proposed that the *recA* protein may change the conformation of normally duplex molecules to a conformation prepared for homologous pairing. In addition, Shibata et al. (167, 168), found that the *recA* protein promotes the homologous pairing of single-stranded DNA with double-stranded DNA to form D loops. This would also be expected to be an intermediate step in any recombinational process.

REPAIR MECHANISMS

Excision Repair

The history of research on excision repair in

phage T4 and a description of many of the functions of endonuclease V (a key enzyme in this pathway) have been presented by Friedberg (55). Excision repair in phage T4 is known to operate on thymine-containing pyrimidine dimers, bringing about their removal from acid-insoluble DNA (138, 163). Endonuclease V, the product of the *den*V gene, catalyzes the first (40a, 143, 157, 203) and second (40a, 203) steps of this pathway and is also involved in repair of heteroduplex loops in DNA (12). It has been suggested by Ebisuzaki et al. (48) that MMS-induced lesions, which depend on a pathway involving the host *polA* function, also may be subject to a form of excision repair. Thus far, no other type of lesion has been identified as being subject to excision repair in phage T4.

In recent experiments on phage T4, the average patch size of newly synthesized DNA undergoing excision repair of UV-induced lesions was measured by using the bromodeoxyuridine-photolysis technique developed by Regan et al. (150). The average patch size was found to be approximately four nucleotides long (D. Yarosh and B. Rosenstein, personal communication). This compares with average short-patch excision repair lengths of 16 to 24 nucleotides (100) and 30 nucleotides (153) in *E. coli* and approximately 100 nucleotides in human cells (149).

Although in vitro endonuclease V makes a number of incisions about equal to the number of dimers in the DNA (56), in vivo only approximately 50% of all thymine dimers are removed (138). Possibly, there are neighborhood effects which restrict the action of the excision repair pathway in vivo.

Excision repair of dimers seems to operate only during part of the infectious cycle. From tbe data of Pawl et al. (138), it appears that maximum dimer removal occurs by about 5 min after infection. Using a temperature-sensitive *den*V mutant in a temperature shift experiment, Sato and Sekiguchi (154) showed that endonuclease V was required at early times, but that it was not used in repair by 19 min after infection under conditions where DNA replication began at 12 min.

In addition to *den*V, five other genes were identified as being involved in excision repair in phage T4 by Ebisuzaki (47) and by Maynard-Smith and Symonds (120) through the use of survival-of-phenotype experiments. These are genes 1, 30, 42, 45, and 56, which code for deoxyribonucleotide kinase, DNA ligase, deoxycytidylate hydroxymethylase, a component of the DNA replication complex, and deoxycytidine-deoxyuridine di- and triphosphatase, respectively, (211). Furthermore, the host *polA* product (polymerase I) participates in this pathway (121, 138, 199).

Although the *E. coli* dimer incision enzymes are present initially in phage T4 infections, they play no role in incision of the phage DNA. Wallace and Melamede (200) found no difference in the UV sensitivity of irradiated wild-type phage T4 when it was grown on wild-type *E. coli* and when it was grown on an *E. coli uvrA* strain. Irradiated *den*V phage also showed no difference in sensitivity on these two host strains. Strike (183) showed that infection of *E. coli* with phage T4 or phage T5 results in inactivation of the host-specified UV-specific endonuclease, probably by newly synthesized proteins in each case. Pawl et al. (138) showed that *E. coli* exonuclease V, which theoretically could act in excision repair, had no apparent effect. These workers also showed that a phage-encoded 5′-to-3′ exonuclease, which also theoretically could be involved, was expressed at a time when no additional dimers were removed. The host exonuclease V was shown by Behme et al. (10a) to be 100% inhibited in a noncatalytic reaction with a protein synthesized after phage T4 infection.

The limitations of excision repair in phage T4 are interesting to enumerate. This process acts on a limited subgroup of the pyrimidine dimers induced by UV irradiation, is limited in its time of expression, and is limited in the enzymes which can participate. How some of these limits are created at the molecular level is unknown.

Homologous Chromosome-Dependent Repair

Multiplicity reactivation. As discussed above, MR is a form of repair that occurs when two or more damaged homologous phage chromosomes are present within a bacterium. This process is effective in repairing lesions caused by eight of the nine inactivating agents tested in phage T4 (Table 2). The lesions that can be overcome by MR apparently include double-strand breaks produced by ^{32}P decay, the largely single-strand lesions caused by alkylating agents or UV light, and the cross-links produced by PUVA. Thus, MR seems to be effective against a wide range of lesions.

Table 3 lists 12 genes, each of which is required for MR of phage T4 treated with at least one of the five inactivating agents tested. These genes are genes 32, 41, 44, 46, 47, 59, *den*V, *uvs*W, *uvs*X, *y* of phage T4 and genes *polA* and *recA* of the host. Table 4 lists the seven of these genes which are also required for spontaneous recombination in phage T4. Five of these genes (genes

TABLE 3. *Genes required for MR of phage T4 treated with various inactivating agents*

Gene	MR requirement[a]				
	HNO_2[b]	MMC[c]	UV irradiation	EMS[d]	MNNG[e]
32	+	+	+ (40)[f]	NT[g]	NT
41	NT	NT	+ (39, 193)	NT	NT
44	NT	NT	+ (40)	NT	NT
46	+	+	+ (40)	0	0
47	+	+	+ (40, 136)	0	0
59	NT	NT	+ (40)	NT	NT
*den*V	0	0	+ (68, 185, 192)	NT	NT
*uvs*W	NT	NT	+ (185, 191)	NT	NT
*uvs*X	+	0 or +[h]	+ (27, 68, 185, 192)	NT	NT
y	+	+	+ (27, 185)	NT	NT
Host *polA*	NT	NT	+ (121)	NT	NT
Host *recA*	NT	NT	+ (142)	NT	NT

[a] +, This gene is required for MR, as determined by the decrease in MR shown by a mutant defective in the gene when compared with the MR of wild-type phage; 0, this gene is not required for MR, as determined by the finding that a mutant defective in the gene showed an MR level equal to or greater than the wild-type level.
[b] See reference 136.
[c] See reference 78.
[d] See reference 88.
[e] See reference 155.
[f] Numbers in parentheses are reference numbers.
[g] NT, Not tested.
[h] In this case MR was measured by DNA synthesis in multicomplexes (160) rather than by survival of infective center-forming ability of multicomplexes as in all other cases.

TABLE 4. *Genes of phage T4 required for MR and recombination and their functions*

Gene	Function	References to experiments showing requirement of gene function for recombination
32	Helix-destabilizing protein, binds to single-stranded DNA (2)[a]	136
46	Exonuclease (140, 204)	18
47	Exonuclease (140, 204)	18
59	Unknown, mutants have DNA arrest phenotype similar to gene 46 and 47 mutants (165)	165
*uvs*W	Unknown	64, 191
*uvs*X	Unknown	69
y	Unknown	27

[a] Numbers in parentheses are reference numbers.

32, 46, 47, *uvs*X, and *y*) are required for MR of HNO_2-, MMC-, and UV-treated phage (Table 3). The other two genes (genes 59 and *uvs*W) are needed for MR of UV-treated phage, but have not been tested with respect to the other agents.

These results suggest that MR of phage treated with HNO_2 or MMC occurs by a repair pathway that has at least five steps in common with spontaneous recombination. For UV-treated phage there are at least seven common steps. In contrast, MR of EMS- or MNNG-treated phage does not require the products of genes 46 and 47 (Table 3). Thus, it appears that there are at least two pathways of MR, one which requires these two recombination functions and one which does not. The five genes which are required for MR (Table 3) but are not known to be needed for phage recombination are genes 41, 44, and *den*V of the phage and genes *polA* and *recA* of the host.

When a genetic cross is carried out between differentially marked phage, a higher frequency of recombination between the markers is usually found if the phage are first treated with an inactivating agent than if they are untreated. The percent increases in recombination in multiple infections with different treatments can be given in a uniform way if doses are expressed in terms of lethal hits to wild-type phage (in single infections). The increases in recombination per lethal hit were calculated for a number of inactivating agents from data in the literature (58). These values are 15% for ^{32}P decay (186), 22% for X rays (67), 41% for EMS (88), 43% for UV irradiation (49), 140% for HNO_2 (58), and 660% for MNNG (155). Presumably, these increases in recombination reflect recombinational repair which goes on during MR.

In the earliest work by Luria (110) it was reported that the presence of many UV-damaged genomes of a T-even phage within a cell did not affect the ability of a single undamaged

T-even genome in the same cell to carry out a successful infection and produce viable progeny. This was confirmed by Luria and Dulbecco (112) for multiplicities of infection of less than five. These results imply that UV-induced lethal damages are not recombined into functional DNA. However, there is some recombination between damaged and undamaged chromosomes, since if highly damaged chromosomes are allowed to infect cells along with undamaged chromosomes, genetic markers (42) and single-stranded DNA fragments (166) from the damaged chromosomes can be rescued and appear in progeny phage.

Masker and Kuemmerle (116) showed with an in vitro system using phage T7 DNA that recombinants are formed between UV-damaged and homologous nondamaged chromosomes. These recombinant chromosomes are taken up by in vitro encapsidation to produce viable phage. Thymine dimers were shown to be absent from these recombinant chromosomes. These authors concluded that damaged sites are not recombinationally exchanged into undamaged molecules at a measurable rate.

Rayssiguier and Vigier (147, 148) analyzed the progeny produced from individual single bursts when a cross was performed between differentially marked phage that had been UV irradiated. Under the conditions used by these workers the progeny produced were largely the products of successful MR. In the first experiment of Rayssiguier and Vigier (147) the parental phages differed from each other by markers at 26 well-distributed sites. In 23 of 34 single bursts, a clone of progeny phage with either one or the other of the parental combinations of markers was found. In the second experiment (148), 35 markers were used to differentiate the parents, and 28 to 31% of the single bursts contained a progeny clone with one or the other parental genotype. The single bursts also contained different recombinant types. About 95% of the observed recombinants occurred only once in a burst. These results suggest that during MR of UV-damaged phage there is an early appearance in a fraction of the bacteria of an undamaged parental genome which is able to replicate and there is also the subsequent formation of many recombinants.

MR has been shown to occur with UV-treated phage λ (90) and to produce a larger effect when excision repair is absent (5). MR of phage λ depends on both host and phage recombination functions (85).

MR of UV-treated phage has been found in a number of systems in addition to phages T4 and λ. These include phages T2, T5, and T6 (110, 112), phage T1 (190), and Vi-phage II of *Salmonella typhi* (15). Also, phage φX174 treated with proflavine plus light can undergo MR (140). With UV-treated phage T7, general MR has not been found (110, 112), although cross-reactivation, or marker rescue, was demonstrated in vivo (32) and elimination of thymine dimers from encapsidated recombinant DNA formed in vitro was reported (116), as described above.

The MR pathway which acts on UV-induced lesions in phage T4 infections is apparently error free since it overcomes lethal lesions without introducing new mutations (217). Similarly, the MR of phage φX174 exposed to proflavin plus light was shown to be recombinational in nature, but not mutagenic (140).

In summary, MR acts on many types of DNA lesions. One type of MR appears to involve recombinational processes since it depends on gene functions required for spontaneous recombination and occurs in association with increased recombination between genetic markers. In addition, several lines of evidence indicate that MR acts specifically to circumvent UV-induced lesions, but does not transfer such lesions from damaged to undamaged DNA. A variety of different types of phage are capable of MR, suggesting that it is a fundamental process. Also, it does not seem to be an error-prone form of repair.

Prophage reactivation. When lysogenic phage treated with a DNA-damaging agent are allowed to infect host cells carrying homologous heteroimmune prophage, survival of infective center-forming ability is characteristically higher than after a similar infection of a nonlysogenic host or a host carrying a nonhomologous prophage. This phenomenon is referred to as prophage reactivation. A comprehensive review of phage λ prophage reactivation was presented in 1975 by Devoret et al. (41). Prophage reactivation of UV-treated phage λ repairs 50% of the lethal lesions, either those remaining when another repair process (host cell reactivation) is also occurring or those present when host cell reactivation is inactive. Prophage reactivation depends on the host *recA* gene product and, to a lesser extent, on the λ *red* gene product. Other bacterial *rec* gene functions, such as *recC*, are dispensable. Prophage reactivation is not mutagenic (21). In addition to prophage reactivation in phage λ, a type of prophage reactivation has been reported for phage P22 after treatment with nitrogen mustard or UV irradiation and growth in *Salmonella typhimurium* lysogenic for P221*b* (214).

Cross-reactivation. Cross-reactivation, or marker rescue, can be observed when cells are

jointly infected by phage differing at one or more genetic loci and when the phage of one genotype are treated with an inactivating agent and the other phage are not. Under these conditions the genetic markers of the inactivated phage can be recovered (or rescued) in the progeny phage, which nevertheless derive most of their genes from the undamaged parent. Cross-reactivation appears to reflect the action of a repair process that redistributes genetic material, but does not necessarily result in a damaged chromosome being restored to an undamaged state. The cross-reactivation of markers from UV-inactivated phage T4 was studied by Doermann (42). He found that the average clone size of rescued genetic markers in single infections was about two. The transfer of genetic information from damaged chromosomes to surviving progeny probably occurs by transfer of a single-stranded patch of DNA, since UV-damaged DNA was shown not to replicate and single-stranded patches from UV-damaged DNA were found to be transferred to replicating DNA (166).

Burck et al. (33) showed that in infections by UV-treated phage T7 there was a correlation between replication of a genetic region and the probability that a marker in that region would be rescued. Replication apparently was initiated at various sites near a position located at 17% of the total length from the left end of the phage T7 chromosome and then continued until a thymine dimer was encountered. There were then more copies of the region near the site of initiation of DNA replication than of more distant regions. This explains why after UV irradiation markers distributed about a point 17% of the total distance from the left end of the chromosome could undergo marker rescue most efficiently. Presumably, a similar explanation involving four sites of initiation could explain the data of Womack (210) for phage T4, since four regions of the genome were characterized by high levels of marker rescue after UV irradiation.

Other interactions between homologous chromosomes. There have been several other studies of the interactions between damaged and undamaged phage chromosomes when both are present in the same cell. Although these studies were not specifically undertaken to elucidate repair processes, they nevertheless contribute to our understanding of recombinational repair and thus are reviewed here.

In a situation where one phage λ genome is present as a prophage chromosome and a second λ genome, carrying PUVA lesions, is introduced as a homoimmune, nonreplicating chromosome, considerable genetic recombination occurs (101). This recombination is dependent on the *recA*, *uvrA*, and *uvrB* genes of the host cell, but not on the *recB*, *recC*, *recF*, and *lexA* genes. In further work, *E. coli* cells lysogenic for phage λ were infected with additional λ phage and incubated to permit the newly infecting DNA to form covalently closed circles. When these cells were then superinfected with PUVA-treated phage, many of the circular molecules were cut (151). This process, in which undamaged DNA is cut because of the presence of homologous damaged DNA, is called cutting in *trans*. It was also shown (152) that undamaged phage 186 DNA could be cut in *trans* in the presence of damaged phage 186 DNA, but not in the presence of nonhomologous damaged phage λ DNA. Similarly, damaged phage λ DNA caused undamaged λ DNA, but not undamaged phage 186 DNA, to be cut in *trans*. Thus, DNA must be both homologous and damaged in order to promote cutting in *trans*. Similar experiments have also been carried out in vitro. Cassuto et al. (34) showed that undamaged, supercoiled, circular φX174 DNA could be cut in *trans* in the presence of damaged φX174 DNA, but not in the presence of damaged ColE1 DNA. However, damaged ColE1 DNA could induce cutting in *trans* of homologous undamaged ColE1 DNA. It has been proposed by Ross and Howard-Flanders (152) that cutting in *trans* is the result of a cut-pair-cut sequence of events; that is, a cut is made in the damaged chromosome at the site of damage, pairing occurs between the damaged and undamaged chromosomes, the free end of the cut strand from the damaged chromosome invades the undamaged chromosome, and a cut is induced in the undamaged chromosome as part of an overall recombination process. It can be speculated that this sequence of events generally occurs in recombinational repair of a damaged site.

Postreplication Recombinational Repair

In *E. coli*, a type of repair has been described which occurs after replication of damaged DNA and which depends on functions also required for genetic recombination. This postreplication repair process has been characterized both biochemically and genetically, as reviewed by Howard-Flanders (80). It is thought that during replication of UV-damaged DNA, gaps are formed in newly synthesized strands opposite the lesions in the parental strands. These gaps are thought to be filled by recombination (i.e., physical transfer of strands) between daughter chromosomes. The products of genes *recA* and *recF*, but not the products of genes *recB* and *recC*, seem to be involved.

The plaque-forming ability of phage T4 upon single infection is inactivated by treatment with a number of the DNA-damaging agents listed in Table 1. This table also indicates the extent to which various phage mutants are more sensitive than the wild type to these agents. The increased sensitivities of the mutants presumably reflect reduced repair. Mutants defective in genes 32, 46, 47, 59, *uvs*W, *uvs*X, and *y* (the genes whose functions are involved in genetic recombination and MR [Table 4]) are among the mutants most sensitive to the inactivating agents tested. This suggests that one or more recombinational repair processes are important even in single infections. Presumably, any recombinational process carrying out repair of a singly infecting chromosome would act after replication, when two daughter chromosomes have been formed. Thus, such repair has been inferred to be analogous to postreplication recombinational repair in *E. coli* (136). Using a survival-of-phenotype test, Maynard-Smith and Symonds (120) have shown that genes 1, 30, 32, 41, 42, 43, 44, 45, and 56 are all involved in the same repair pathway as gene *y*. These workers were unable to test some of the most interesting genes whose products are required for recombination, such as genes 46 and 47, because amber mutants having defects in these genes had high transmission coefficients on Su^- hosts, and this interfered with the test. Since the products of all of the genes which were tested are necessary for phage DNA synthesis, it was concluded that *y* repair must depend on normal phage DNA synthesis. Thus, these results also imply that *y* repair is a postreplication repair process. Evidence has also been presented that gene 58-61, whose product is used in DNA synthesis (105, 171), acts in this pathway (64). Thus, seven genes required for recombination (genes 32, 46, 47, 59, *uvs*W, *uvs*X, and *y*), as well as nine additional genes required for DNA synthesis (genes 1, 30, 41, 42, 43, 44, 45, 56, and 58-61), may be involved in one or more postreplication recombinational repair processes in phage T4. The products of genes *uvs*X and *y* appear to act in a pathway separate from the *den*V pathway, which carries out excision of thymine dimers, since double mutants defective in *uvs*X and *den*V or *y* and *den*V are more sensitive to UV inactivation than *uvs*X, *y*, or *den*V single mutants (26, 185).

Error-Prone Repair

Extensive work has been performed on error-prone repair in *E. coli* in response to UV-induced lesions. This has been reviewed by Witkin (209).

In phage T4, the hypothesis that UV mutagenesis occurs by error-prone repair is an attractive explanation for a number of observations. Although the premutagenic lesion introduced by UV irradiation in phage T4 is the thymine dimer, the transitions that arise in response to UV irradiation are mainly guanine-cytosine to adenine-thymine, indicating that the thymine dimers stimulate a length of repair synthesis longer than the initial lesion (126). UV mutagenesis is eliminated by a gene 30 *ts* ligase mutation at 31°C (216), a temperature at which the burst size of the mutant is still about 35% of the wild-type value (18). This implies that an error-prone repair pathway can be blocked independent of effects on replication. The alleles *px*, *y*, and 1206, which are defective in genes *uvs*X, *y*, and *uvs*W, respectively, have been shown to inhibit UV mutagenesis, and it has been proposed that these genes encode functions necessary for error-prone repair (62). Recently, Yarosh et al. (217) showed that the gene 43 polymerase antimutator alleles *ts*CB120 and *ts*CB87 block both UV mutagenesis and PUVA mutagenesis under conditions in which the phage grow well. It was proposed that the gene 43 polymerase is employed in error-prone repair synthesis in addition to its function in chromosome replication.

Fidelity of replicative DNA synthesis by the phage T4 DNA polymerase is thought to depend on the specificity of the polymerizing function of the enzyme and on the efficiency of its 3′-exonuclease editing function (60, 108). Alberts and Sternglanz (1) have argued that much of the enzymatic complexity of replication may be attributed to processes which promote fidelity. Liu et al. (105) observed that replication fidelity was increased when the protein products of genes 32, 44, 45, and 62 were present along with the gene 43 polymerase in a cell-free reaction mixture. These workers found that the increase in fidelity was accompanied by an increase in deoxyribonucleotide turnover and suggested that the four additional proteins may act to enhance the efficiency of the 3′-to-5′ exonuclease proofreading by the DNA polymerase.

Working with *E. coli*, Villani et al. (198) have proposed that UV induction of mutations depends upon the relaxation of the polymerase proofreading activity. In accord with this proposal, the results of Yarosh et al. (217) showing that two antimutator polymerase alleles of phage T4 inhibit UV mutagenesis imply that the altered polymerases may be unable to relax their proofreading sufficiently for UV mutagenesis to take place. However, these results are also consistent with the interpretation that the antimutator polymerases have enhanced discrimination in initially incorporating complementary nucleotides.

Additional data from Mufti (134) on phage T4 indicated that although UV mutagenesis is blocked by the antimutator polymerase allele *ts*CB87, it is restored or even amplified if a second allele of any of genes 32, 41, 44, and 45 is present. The products of these genes are components of the phage replicative complex (105). It is not known whether the *ts*CB87 antimutator polymerase is more stringent in its initial base selection or whether it has an excessive 5'-to-3' exonucleolytic activity compared with the wild-type polymerase. Whichever may be the case, the altered products of genes 32, 41, 44, and 45 influence the antimutator polymerase to carry out the DNA synthesis associated with UV mutagenesis much less accurately. Thus, it appears that error-prone repair of UV-induced lesions is carried out by a complex of proteins similar to the replicative complex.

In phage λ, UV mutagenesis occurs by a pathway that appears to be equivalent to SOS repair in the *E. coli* host (41, 209). This mutagenesis depends on the host genes *recA*, *lexA*(*exr*), and *lexB*(*zab*). Treatment of host cells with UV irradiation before infection by phage λ causes enhanced expression of this mutagenic pathway.

HNO_2 mutagenesis in phage T4 may also occur by error-prone repair. Early work on HNO_2 mutagenesis was generally interpreted in terms of DNA lesions which cause mispairing upon replication (9, 53, 62, 189, 196, 197). However, recently (17) it was found that all detectable HNO_2 mutagenesis was eliminated by a gene 30 *ts* ligase allele at 32°C, a semipermissive growth temperature for the mutant. Thus, effects on HNO_2 mutagenesis appear to be separable from effects on replication. Drake and Greening (43) showed that the antimutator polymerase alleles *ts*CB87 and *ts*CB120 could eliminate virtually all HNO_2-induced transitions from adenine-thymine to guanine-cytosine at two sites examined. Since gene 43 polymerase alleles, as well as a gene 30 ligase allele, can eliminate HNO_2 mutagenesis, both of these enzymes appear to be employed in the same mutagenic pathway. It was proposed that HNO_2 mutagenesis occurs by an error-prone pathway which includes the gene 30 ligase and the gene 43 polymerase (17).

Kerr and Hart (92) showed that HNO_2 mutagenesis in phage λ occurs if treated phage are allowed to multiply in UV-irradiated wild-type bacteria. No HNO_2 mutagenesis was observed if the treated phage multiplied in unirradiated bacteria. The HNO_2-treated phage did not undergo mutagenesis if UV-irradiated *recA*$^-$ or *exr*$^-$ (*lexA*$^-$) bacteria were used. These results imply that HNO_2 mutagenesis occurs by a UV-inducible error-prone repair pathway which depends on the functions of the *recA* and *exr*(*lexA*) genes. Kerr and Hart also showed that HNO_2-treated phage have a much-increased survival when grown in UV-irradiated bacteria compared with unirradiated bacteria and that this UV reactivation does not depend on the *recA* or *exr*(*lexA*) function. Therefore, in phage λ HNO_2-induced premutational lesions and prelethal lesions appear to be acted on by separate repair pathways.

The *recA* and *exr*(*lexA*) functions are also necessary for mutagenesis by 4-nitroquinoline-1-oxide, MMC, and X rays in *E. coli* (87, 209). Mutagenesis by these agents is thought to occur by an inducible error-prone repair process. Here too, the mutagenic process can be separated from the repair of prelethal lesions (28).

The error-prone repair of premutagenic UV- and HNO_2-induced lesions in phage T4 may either be a minor pathway or be replaceable by other repair pathways. When a gene 30 mutation blocks HNO_2 mutagenesis or when a gene 30 or gene 43 mutation blocks UV mutagenesis in phage T4, there is not much more sensitivity to the lethal effects of the agent (17, 216, 217).

It was reported by Pietrzykowska (139) that in phage λ, mutagenesis by 5-bromodeoxyuridine depends on the *E. coli recA* function and the phage *red* function. Since these are repair functions, she proposed that this mutagenesis occurred through error-prone repair. However, Hutchinson and Stein (86) carried out similar experiments using 5-bromouracil, and they were unable to find any dependence of mutagenesis in phage λ upon *recA* or *red* function. They found a nonlinear dependence of the number of mutations induced on 5-bromouracil incorporation. There was also an increase in the frequency of mutant heterozygotes among progeny. The results of these authors implied that 5-bromouracil mutagenesis is a consequence of base mispairing occurring during replication and fit with a model in which 5-bromouracil is recognized when it occurs in a mispair and is removed by a mismatch repair process that acts at low levels of incorporation of the analog.

Photo-Reactivation

Photo-reactivation of DNA consists of the enzyme-mediated, light-dependent monomerization of pyrimidine dimers, resulting in repair of the DNA and restoration of its biological integrity (184). Some of the earliest work on photo-reactivation was performed with phage. In 1949, Dulbecco showed that photo-reactivation existed for phage of the T series (44). In 1950 Dulbecco further showed that a host enzyme was likely to contribute to photo-reacti-

vation, since this process could be demonstrated for phage T2 as early as 10 s after adsorption (45). The action spectrum for this photo-reactivation had a maximum at about 365 nm. The photo-reactivable sector varied from 0.20 for phages T4 and T5 to 0.68 for phage T1. As shown by Harm (68), the size of the photo-reactivable sector depends on the overlap of photo-reactivable damages with damages reactivable by other pathways, such as the *den*V excision pathway. The magnitudes of the photo-reactivable sectors indicate that photo-reactivation is a major repair mode for UV-induced damage.

Photo-reactivation is usually measured in terms of increased viability after exposing a population of UV-inactivated organisms to visible light, as done by Dulbecco (44, 45) and Bowen (23, 24). However, splitting of thymine dimers can also be measured. Helene et al. (74) found that the phage T4 gene 32 protein can promote the photosensitized splitting of thymine dimers in vitro. It is not known whether this reaction or possibly other similar reactions carried out by tryptophan-containing proteins which bind to DNA (73) are of significance in repairing pyrimidine dimer-containing DNA in vivo.

Photo-reactivation of phage T2*r* DNA within infected cells was shown to consist of distinct light reaction and dark reaction steps, each of these steps following first-order kinetics (23, 24). These conclusions were reached in experiments in which a series of short high-intensity light flashes were used. It was found that the dark reaction occurred first, followed by an irreversible light reaction.

Host Cell Reactivation and Host Cell-Mediated Ultraviolet Reactivation

Devoret et al. (41) have provided a comprehensive review of both host cell reactivation and host cell-mediated UV reactivation (Weigle reactivation) as a response to UV-induced damage in phage λ. Witkin (209) has briefly reviewed UV reactivation in phage λ. Both host cell reactivation and UV reactivation of UV-irradiated phage λ are dependent entirely on host cell functions. Host cell reactivation is an excision pathway which can repair 85% of UV-induced lethal lesions, is not mutagenic, and depends on at least the four host genes *uvrA*, *uvrB*, *uvrC*, and *uvrD*. It takes place before phage DNA replication.

UV reactivation (or Weigle reactivation) is the phenomenon of enhanced survival of phage treated with an inactivating agent, when the host cell has been exposed to a low UV dose before infection. For UV-treated phage λ this process depends on the products of the host genes *recA*, *lexA*(*exr*), and *lexB*(*zab*) and can repair 50% of the lethal lesions (most of which can also be repaired by host cell reactivation, if present).

Using PUVA as an inactivating agent, Belogurov et al. (11) showed that host cell reactivation of phage λ in single infections requires the functions of host genes *uvrA* and *lexA*(*exr*). They also showed that UV irradiation of the wild-type bacterial host before single infection with PUVA-treated phage λ caused increased resistance to the lethal effects of PUVA (i.e., UV reactivation of the PUVA-treated phage). This UV reactivation did not occur in *E. coli* mutants defective in gene *uvrA*, *recA*, or *lexA*(*exr*). Thus, UV- and PUVA-induced lesions in phage λ appear to be repaired by pathways of host cell reactivation and UV reactivation which require some common gene functions.

Phage T7 which has been treated with UV light or PUVA can also undergo host cell reactivation. In the case of UV irradiation, the *E. coli uvrD* function is known to be required (94), and in the case of PUVA, the *E. coli uvrA* function is required (11). In addition, phage T1 damaged by UV light has been shown to undergo host cell reactivation which depends on the functions of *E. coli* genes *uvrA*, *uvrB*, and *uvrC* (81).

Other Pathways of Repair

Ebisuzaki et al. (48) suggested the existence of three separate pathways for repair of MMS-treated phage T4 based on a study of the sensitivities of double mutants compared with the sensitivities of single mutants. The three pathways were (i) the *uvs*X-*y* pathway, (ii) a pathway requiring the *E. coli polA* gene function, and (iii) a pathway requiring the function of the phage *mms* gene. These authors also studied sensitivities to UV treatment and proposed that the same three repair pathways act on UV-induced lesions. In addition, their results indicated that the host *polA* and phage *den*V gene products act in the same pathway in repairing UV-induced lesions. As discussed above, the *uvs*X-*y* pathway is thought to be analogous to postreplication recombinational repair, and the *den*V-host *polA* pathway has been shown to be an excision repair pathway. The pathway dependent on gene *mms* is apparently an additional one.

Using similar criteria, van Minderhout et al. (193, 194) obtained evidence for a third repair pathway for UV-induced lesions, which may or may not be the same as the gene *mms*-dependent pathway. These workers used two mutations, *uvs*58 and *uvs*79, which caused sensitivities to UV that were more than additive with the sen-

sitivities caused by the *den*V mutation or the *y* mutation or both. These mutations are in gene 41 (39, 194). As described above, the gene 41 product is a ribonucleic acid primase involved in DNA replication. The mutants *uvs*58 and *uvs*79 have effects on DNA synthesis (194), so the pathway which they define was called replication repair (39).

DNA STRUCTURES PROBABLY INVOLVED IN REPAIR

Nicked DNA

When *E. coli* cells lysogenic for phage λ are superinfected by phage λ, the infecting linear phage chromosomes become covalently closed circles that upon extraction show a fast-sedimenting behavior, implying a superhelical structure. When such superinfected cells were treated with MMS, the superhelical, closed circular chromosomes acquired single-strand nicks (25). After removal of the MMS, additional incubation allowed the nicks to be repaired. Isolated superhelical circular chromosomes exposed to MMS in vitro acquired nicks if a heat-labile factor from extracts of *E. coli* was present. The cleavage and rejoining of the phage λ DNA were thought to reflect an excision-like repair process (25).

Similarly, when phage T4 was treated with MMS and then allowed to inject its DNA into *E. coli* cells, single-strand nicks were produced in the DNA. Such nicks were removed if the phage used was wild type, but were not removed if gene 59 mutant phage was used (212). The nicked DNA is presumably an intermediate in a repair pathway which involves the gene 59 product.

After UV treatment of phage and injection of the DNA into the host cell, it was expected that nicks would occur in the DNA by the action of endonuclease V during excision repair, as discussed above. Direct measurements of nicks after UV treatment were made, but under conditions where some phage growth had occurred intracellularly before UV treatment. When phage T4 was allowed to inject its DNA and 6.5 min later the infected cells were treated with UV irradiation, the phage DNA became nicked (212). These nicks could be repaired in the presence of a wild-type genome, but not if a mutation in gene 59 was present (212). In other experiments, Wakem and Ebisuzaki (199) examined DNA from phage T4-infected cells wbich had been incubated in growth media for 30 min at 25°C, UV irradiated, and incubated further in growth media. When a functional *den*V product was present, nicks were rapidly induced and then subsequently repaired. When the *den*V product was defective, nicks were produced more slowly, over a period of about 15 min, and these nicks were also repaired.

As discussed above, nicks are made in *trans* within an otherwise undamaged chromosome when a homologous damaged phage chromosome is present in the same cell. These nicks are presumably induced during a recombinational repair process.

Single-Strand Gaps

Prashad and Hosada (141) have shown that the products of phage T4 genes 46 and 47 catalyze the formation of single-strand gaps from nicks. Since, as described above, mutants defective in either gene 46 or gene 47 have a reduced ability to carry out MR and probably also postreplication recombinational repair, it is likely that DNA molecules with single-strand gaps are intermediates in these repair processes. The gene 43 polymerase of phage T4 can participate in repair of gaps in recombinant molecules (129) and may thus also be used in other repair pathways which involve gapped DNA.

In general, the evidence reviewed above for the occurrence of single-strand nicks would not have distinguished between nicks and gaps. Therefore, some of those results may reflect the formation of gaps as intermediates in DNA repair.

Supercoiled DNA

As reviewed by Radding (145), a number of reports have indicated that supercoiling of duplex DNA promotes uptake of homologous single strands. These events are seen as D loops by electron microscopy. This kind of uptake would be a logical step in recombinational repair. The *E. coli recA* protein can also promote the uptake of DNA single strands into either relaxed or superhelical DNA (124, 167). Thus, in recombinational repair there could be a requirement for DNA supercoiling or for a protein with the function of *recA* or for both of these.

McCarthy (122) proposed that in phage T4 infections the products of genes 39, 52, and 60 either form a new DNA gyrase or alter the host gyrase (topoisomerase II). As described above, such an enzyme can alter the superhelical state of DNA. Liu et al. (106) and Stetler et al. (180) have isolated a protein complex from phage T4-infected cells which appears to be composed of the products of genes 39, 52, and 60 and has type II topoisomerase activity. Although the biologically important reaction catalyzed by this complex in vivo has not been determined, it is thought to alter the topological state of DNA during the initiation of phage T4 DNA replica-

tion (122, 123). As described above, the phage T4 topoisomerase appears to be needed for recombinational repair of PUVA- and MMC-induced lesions. Similarly, the *E. coli* gyrase appears to be needed for repair of UV-induced lesions in phage λ. Thus, it is probable that the supercoiled state of DNA affects repair.

Branched Intermediates

Broker and Lehman (30) and Broker (29) showed by electron microscopy that branched DNA structures are intermediates in spontaneous recombination. These are probably most often the result of branch migration. Since in general, the enzymes of phage T4 required for spontaneous recombination are also required for recombinational repair (Table 4), it is likely that there are many steps in common between the two processes. Thus, branched structures are likely intermediates in recombinational repair.

DNA-Protein Complexes

Huang and Buchanan (82) have shown that 19 distinct early proteins produced by phage T4 bind to DNA with various affinities. These include the products of genes 30, 32, 39, 43, 46, and 52, each of which appears to be employed in repair, as discussed above. At present, protein-DNA complexes in phage T4 have not been investigated from the point of view of repair. However, knowledge has been accumulating on the function of phage T4 DNA-protein complexes in DNA replication and recombination, as reviewed by Liu et al. (105) and Mosig et al. (133). Since replication and recombination processes are involved in repair, it is reasonable to assume that DNA-protein complexes are also involved in repair.

MANIFESTATIONS OF REPAIR

Recombination

As a basic genetic phenomenon, recombination has been studied intensively since near the beginning of this century. In 1930 Fisher (52) and in 1932 Muller (135) suggested that the selective advantage of recombination arises from its value in generating genetic diversity and bringing together favorable mutations. However, it has been shown recently (e.g., in authoritative books by Williams [208] and Maynard-Smith [118]) that there are fundamental difficulties in explaining the selective advantage of recombination and sexual reproduction solely in terms of variation.

It was proposed recently (16, 19, 20, 115) that spontaneous recombination actually may be, in large part, a reflection of recombinational repair of naturally occurring lesions and that this repair may provide an important selective advantage for the recombination process that is independent of variation. The arguments used were based in large part on the effectiveness with which recombinational repair can circumvent a wide variety of induced lesions in phage DNA.

The Sexual Cycle

Although the sexual cycle varies greatly among diverse organisms, from phages to humans, the main steps of the cycle are probably of general occurrence. These are as follows: (i) two genomes or parts of genomes come together within a shared cytoplasm; (ii) the genomes pair, so that homologous sequences are adjacent; (iii) accurate exchange of genetic material occurs between the two genomes; and (iv) the exchange is followed by separation of the products of the interaction. The steps of the sexual cycle are the same as those presumed to be required for recombinational repair, including MR, a major form of repair in phage systems.

When damage occurs which destroys information over a common sequence in both strands of duplex DNA, the lost information is potentially recoverable from another homologous chromosome by recombinational exchange. Damages affecting both strands at nearby sites can be caused by cross-linking agents, such as HNO_2, MMC, and PUVA, as discussed above. Similar double-stranded lesions may occur naturally in phage DNA through the action of environmental agents, through endogenous processes, or through a combination of both. An example of the latter would be the introduction of thymine dimers by UV irradiation and then the formation of gaps opposite the dimers by replication. If such double-stranded lesions present a significant problem to phage survival, they could provide the selective basis for the evolution of recombinational repair processes in these organisms. Based on such considerations, Bernstein (16) and Bernstein et al. (20) have proposed that the sexual cycle evolved in large part because it promotes efficient recombinational repair of DNA lesions in the germ lines of organisms.

CONCLUSIONS

DNA repair in the well-studied phage systems is surprisingly complex, given the small sizes of these organisms. The presence of excision repair, recombinational repair, and photo-reactivation is well established, and error-prone repair provides an attractive explanation for a range of observations. In phage T4, these processes (except photo-reactivation) are carried out largely

by phage-encoded gene functions, whereas in smaller phages, including phage λ, host gene functions are mainly used. Very diverse types of lesions have been shown to be reparable, indicating the great flexibility of these phage repair systems.

Special insights have emerged through the use of phage systems. For instance, much of our understanding of recombinational repair has followed from experiments on processes such as MR and prophage reactivation. Studies on phage T4 excision repair, carried out both in vivo and *in vitro*, have allowed some of the steps of this process to be defined with precision, and as a result phage T4 endonuclease V is one of the best-understood repair enzymes. Also, experiments with phages have indicated that the level of accuracy of error-prone repair is determined by a protein complex similar in most of its components to the complex that carries out chromosomal replication. However, each of the insights gained has revealed unanswered questions. For the major repair pathways, the nature and sequence of most of the enzymatic steps and the structures of the DNA intermediates at each stage remain to be elucidated.

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